CHAPTER TWENTY

Recent Advances in the Discovery of Dengue Virus Inhibitors

Jeremy Green, Upul Bandarage, Kate Luisi, Rene Rijnbrand
Vertex Pharmaceuticals, Cambridge, Massachusetts, USA

Contents

ABBREVIATIONS

ADE antibody-dependent enhancement
BHK21 baby hamster kidney-21 cells
BVDV bovine viral diarrhea virus
DENV dengue virus
DHF dengue hemorrhagic fever
DSS dengue shock syndrome
ER endoplasmic reticulum
EC_{50} half maximal effective concentration
HCV hepatitis C virus
IC_{50} half maximal inhibitory concentration
JEV Japanese encephalitis virus

Annual Reports in Medicinal Chemistry, Volume 47
ISSN 0065-7743
http://dx.doi.org/10.1016/B978-0-12-396492-2.00020-5

K_i inhibitory dissociation constant
NOAEL no observable adverse event level
NS2B/NS3pro dengue NS3 serine protease with NS2B cofactor
RdRp RNA-dependent RNA polymerase
Vero African green monkey kidney epithelial cells
VSV vesicular stomatitis virus
WEEV Western equine encephalitis virus
WNV West Nile virus
YFV yellow fever virus

1. INTRODUCTION

Dengue virus (DENV) is a mosquito-borne infection that causes significant morbidity and mortality throughout the tropical and subtropical areas of the world.[1] Severe dengue disease (dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS)) was first recognized in the 1950s following epidemics in the Philippines and Thailand.[1] Prior to 1970, only nine countries had experienced severe dengue outbreaks, but in recent decades, the incidence of dengue has dramatically increased, particularly in urban and suburban areas. Today, the disease is endemic in over 100 countries, with severe dengue disease affecting Southeast Asia, Africa, the Caribbean, the South Pacific, and Central and South America.[2] Currently, over 2.5 billion people worldwide are thought to be at risk of contracting this disease and it is estimated that between 50 and 100 million dengue infections occur every year. According to the World Health Organization (WHO), dengue is now a leading cause of hospitalization and death among children in endemic regions, such as Asia and South America, and is now considered a major international health concern.[3]

After malaria, dengue is the most significant mosquito-borne human pathogen and is transmitted by the *Aedes* family of mosquito. The main vector is the urban-adapted *Aedes aegypti* mosquito, but *Aedes albopictus* and *Aedes polynesiensis* have also been implicated in some outbreaks. The global resurgence of dengue has been attributed to an increase in the *A. aegypti* population and its adaptation to urban areas.[1] Mosquito population control has been employed as a mechanism to prevent dengue outbreaks. These efforts have included the eradication of habitat,[4] the use of bacterial pathogens,[5] and the release of sterilized mosquitoes into the natural population.[6] Although these efforts have been shown to reduce the mosquito population, they have not permanently eradicated the mosquitoes.[1,4] In addition, the termination

of mosquito control efforts leads to a rebounding of the mosquito population along with the recurrence of dengue disease.[7] This was observed in Central and South America where the reemergence of dengue during the 1970s was ascribed to the cessation of mosquito control measures.[7]

There are four similar, yet antigenically distinct, serotypes of DENV: DENV1, DENV2, DENV3, and DENV4. Initial infection with any of the serotypes is typically self-limiting with relatively mild symptoms. Resolution of infection occurs within 4–7 days and is associated with a robust innate and adaptive immune response. Recovery provides lifelong immunity against that particular serotype, but cross-immunity with other serotypes is only partial and temporary. A subsequent infection with a different serotype is more likely to result in severe disease,[1] a result of a phenomenon known as antibody-dependent enhancement (ADE). This occurs when cross-reactive non-neutralizing antibodies generated during the primary infection recognize a heterologous DENV during a second infection resulting in an increase in virus uptake into Fc-receptor-bearing cells. This infection of T-cells increases viral replication and triggers the release of proinflammatory cytokines that are thought to be involved in the development of plasma leakage and DHF/DSS.

DENV is a member of the *Flaviviridae* family of viruses, which also includes West Nile virus (WNV), yellow fever virus (YFV), and Japanese encephalitis virus (JEV). Flaviviruses are small-enveloped viruses that contain a single molecule of positive-strand RNA. The RNA genome is approximately 11 kb in length and encodes for three structural proteins (capsid [C], premembrane [PrM], and envelope [E] proteins) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) (Fig. 20.1).

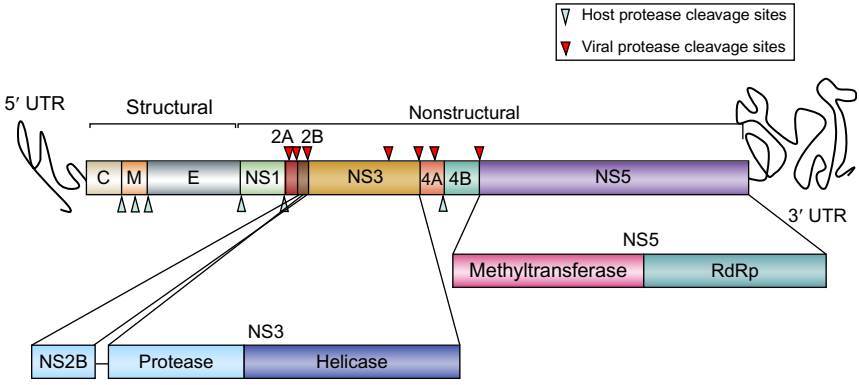


Figure 20.1 Schematic of the DENV genome.

The structural proteins are responsible for the formation and maturation of new viral particles. The nonstructural proteins are required for the replication of the genome, virion assembly[8], and evasion of the innate immune system.[9] Of these proteins, there are only two that are known to have enzymatic activities, NS3 and NS5, making these proteins obvious targets for the development of DENV targeting antivirals. The N-terminal domain of NS3 carries the catalytic domain of the viral serine protease, which, together with the cofactor NS2B, cleaves the viral polyprotein.[10] The C-terminal domain of NS3 contains the helicase,[11] nucleotide triphosphatase (NTPase)[12], and RNA triphosphatase (RTPase)[13] activity of the virus. NS5 contains methyltransferase activity[14] in the N-terminal domain and the RNA-dependent RNA polymerase (RdRp)[15] function in the C-terminal domain. Although these are the only two viral proteins with enzymatic activity, the other nonstructural proteins are also required for proper formation of the replication complex and are also potential antiviral targets.[16–18]

There are currently no therapies or vaccines available against DENV. Multiple efforts are in progress to develop a dengue vaccine, but these vaccines face significant challenges due to targeting multiple serotypes and the cost of producing the vaccine.[19] Currently, the most advanced efforts to develop a dengue vaccine consist of either the combination of live-attenuated strains of each of the four serotypes or the use of chimeric viruses (based on existing licensed vaccines) designed to generate an immune response to either the prM or E protein. Studies have shown that these approaches can generate neutralizing antibody titers to all four serotypes and that the vaccine is relatively safe with low levels of viremia. Interference of the four virus components of the vaccines does occur and it typically requires three vaccinations over a period of several months to achieve neutralizing antibody titers to each of the four serotypes. This raises concerns over whether these vaccines may prove too costly for use in endemic regions with developing economies. It also remains to be seen if the observed levels of neutralizing antibody titers can be maintained or whether boosters are required, as is the case with other flavivirus vaccines.[20]

Drug discovery efforts directed toward DENV have focused on both viral and host targets. The increasing understanding of flavivirus biology and the increasing clinical successes in HCV drug discovery have inspired research efforts applying virtual and high-throughput screening approaches to DENV drug discovery programs. Here we report the most recent advances in anti-DENV medicinal chemistry. Interested readers should also refer to other recent reviews in this rapidly evolving area.[21,22]

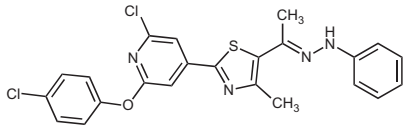
2. VIRAL STRUCTURAL PROTEIN TARGETS

2.1. E protein

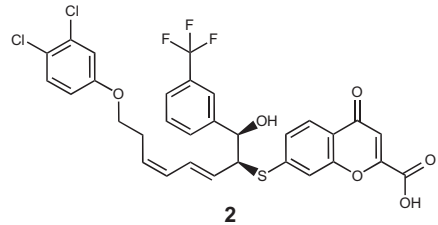
The DENV glycoprotein E forms the outer shell of the flavivirus particle and is responsible for virus–host receptor interactions. Following attachment, the particles are internalized through endocytosis. In the low pH environment of the endosome, the E protein undergoes structural rearrangements that drive the fusion of viral and endosomal membranes prior to release of the viral genome into the cytoplasm.[23,24] Preventing attachment to the host receptor or proper deployment and activation of the E protein fusion domain will prevent infection of permissive cells.

The DENV E protein consists of three structural domains (I, II, and III). The *n*-octyl-β-D-glucoside (β-OG) pocket is a channel buried at the hinge between domains I and II that undergoes a structural change during the fusion activation process. It is highly conserved among flaviviruses and has received attention from several groups.[25]

Virtual screening of 135,000 commercial molecules directed toward the β-OG pocket led to the selection of five molecules for evaluation in Vero cells infected with DENV2.[26] Compound **1** inhibited DENV ($IC_{50} = 1.2$ μM), as well as WNV ($IC_{50} = 3.8$ μM), and YFV ($IC_{50} = 1.6$ μM). Activity in an E protein and pH-specific c6/36 insect cell fusion assay provides evidence that the mechanism of **1** is through inhibition of E protein-mediated cell fusion.



1

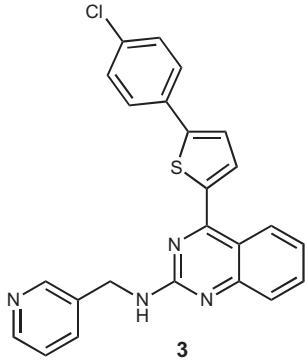


2

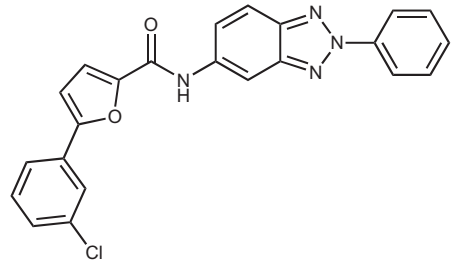
A similar virtual screening approach based on a corporate collection, including natural products, led to the identification of **2**. Compound **2** demonstrated activity in a quantitative DENV2 fusion assay ($IC_{50} = 6.8$ μM) and the antiviral activity was confirmed in DENV2-infected BHK21 fibroblasts ($IC_{50} = 9.8$ μM).[27]

Using a docking study of the β-OG pocket with a 586,829 compound library, two molecules were identified that inhibited DENV2 infection of BHK21 cells.[28] Further SAR development resulted in **3** ($EC_{50} = 0.07$ μM), which is active against all four serotypes of DENV

(EC_{50}s = 0.068–0.496 μM), as well as YFV, JEV, and WNV (EC_{50}s = 0.47–1.42 μM). Biological assays confirmed that **3** bound to viral particles and acted on an early step in the viral life cycle. Additionally, the virus appeared arrested in endosomes, where the E protein normally undergoes a pH-induced conformational change that drives the membrane fusion process.



3



4

An alternate approach, comparing the pre- and postfusion forms of the E protein, identified two sites suitable for small-molecule inhibitor binding.[29] An *in silico* screen was performed using several publicly and commercially available chemical databases. Seven molecules from this screen were tested in DENV2-infected Vero cells and **4** was identified (IC_{50} = 4 μM).

2.2. Peptide fusion inhibitors

A 29-amino acid peptide derived from the DENV2 stem (aa 419–447) targets the postfusion E trimer and blocks pore formation.[30] Surprisingly, the $DV2^{419–447}$ peptide seems to be introduced into the endosome by initially associating with the viral membranes and only associates with the stem during the pH-mediated rearrangement in the endosome. This is driven in part by a relatively hydrophobic C-terminal domain.

Using structural information, the E domain II hinge region was targeted with energy-minimized and structurally stabilized peptides.[31] The two most active peptides identified in this screen contained 20 and 28 amino acids and inhibited DENV2 with IC_{50}s of 7 and 8 μM, respectively. The interaction of the peptides with virions led to surface changes and loss of icosahedral symmetry prior to internalization of the particles. It should be noted, however, that these changes can occur after virus attachment to the cells.

The ability of pre-existing antibodies to enhance DENV uptake into cells was used to develop an assay to test peptide inhibitors for their ability to prevent ADE in K562 cells.[32] Two previously characterized peptides of 33 amino acids[33] and 20 amino acids[31] in length were found to prevent ADE with IC_{50}s of 3 and 6 μM, respectively.

A particular obstacle for the development of DENV entry inhibitors is the possibility that the virus might have multiple paths to enter cells.[24,34,35] This is supported by the observation that carbohydrate-binding agents were able to inhibit infection of some cell types but not others.[34,36] A further complication is that these molecules will have no beneficial effect on already infected cells.

3. VIRAL NONSTRUCTURAL PROTEIN TARGETS

3.1. NS2B/NS3 protease

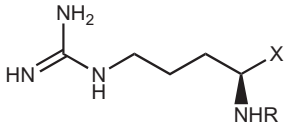
The full-length DENV NS3 protein contains both protease and helicase domains. The N-terminal 180-amino acid residue domain encodes for a serine protease (NS3pro), which requires the NS2B cofactor to be stably folded and to exhibit proteolytic activity.[37] The NS2B/NS3pro complex mediates cleavage of the viral polyprotein (see Fig. 20.1) and possesses a classic serine protease catalytic triad of His51, Asp75, and Ser135. Since NS2B/NS3pro plays a critical role in viral polyprotein processing and replication, it is a promising target for therapeutic intervention for DENV infection.[38]

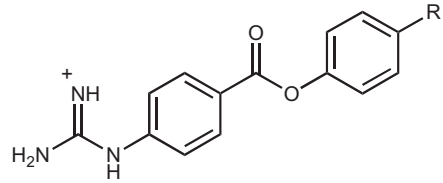
3.1.1 Competitive inhibitors

By analogy to the successful approaches to inhibit the HCV NS3 protease,[39,40] binding of inhibitors to the nonprime subsites of DENV protease provides an opportunity to develop effective small-molecule inhibitors.[21] However, it should be noted that the substrate specificity of NS2B/NS3pro is markedly different to that of HCV NS3/4A protease, while maintaining the drug development challenge of a shallow, solvent-exposed active-site surface. DENV NS2B/NS3pro possesses trypsin-like substrate preference for basic residues (Lys, Arg) at P1, as well as at P2.[41,42]

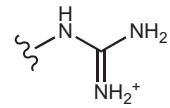
A series of substrate-based, tetrapeptide inhibitors containing various functional groups that can covalently bond with the catalytic Ser has been synthesized and tested against DENV2 NS2B/NS3pro.[43–45] Tetrapeptide boronic acid **5** and trifluoromethylketone **6** have shown good inhibitory potencies toward NS2B/NS3pro with K_is = 0.043 and 0.85 μM, respectively.[43] The substrate-based tetrapeptide aldehyde **7** has also been

reported to have inhibitory potency for NS2B/NS3pro with $K_i = 5.8$ μM,[43] while a truncated but more basic aldehyde **8** was slightly more potent ($K_i = 1.5$ μM).[44]





R = Bz-Nle-Lys-Arg X = $B(OH)_2$ **5**
R = Bz-Nle-Lys-Arg X = $COCF_3$ **6**
R = Bz-Nle-Arg-Arg X = CHO **7**
R = Bz-Lys-Arg X = CHO **8**

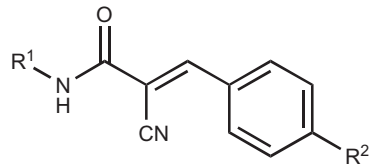
R = 

9

R = CN **10**

An *in silico*, structure-guided, fragment-based design approach to identify DENV protease inhibitors from commercially available molecules led to the identification of compounds **9** and **10**, which inhibited DENV2 NS2B/NS3pro with IC_{50}s = 7.7 and 37.9 μM, respectively.[46]

Arylcyanoacrylamides, better known as early receptor tyrosine kinase inhibitors,[47] have been recently reported as DENV and WNV NS2B/NS3pro inhibitors.[48] The electron density of the aryl group and the central double bond are crucial for the activity while substitution of the amide group did not improve potency. Compound **11** was found to be the most potent inhibitor in this series with $K_i = 35.7$ μM. Possessing low molecular weight and high ligand efficacy,[49] compound **11** was identified as a candidate for further SAR exploration.[48] Combining the arylcyanoacrylamides with retropeptide-based inhibitors has led to **12**, but structural information on binding mode is lacking.[50]

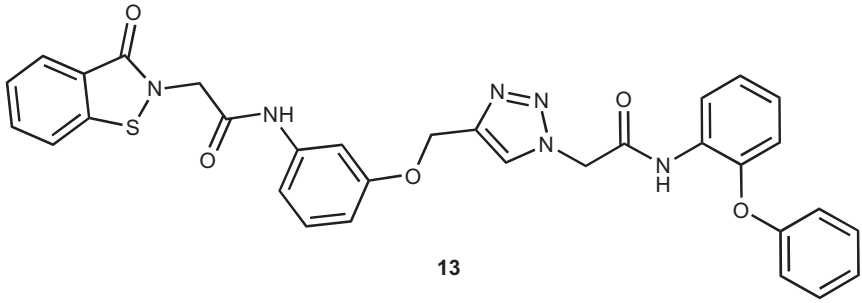


11 R^1 = H; R^2 = OH

12 R^1 = cPr; R^2 = COArg-Lys-Nle-NH_2

A series of triazole-containing benz[d]isothiazol-3(2H)-one derivatives has been identified as inhibitors of DENV2 NS2B/NS3pro.[51] Several

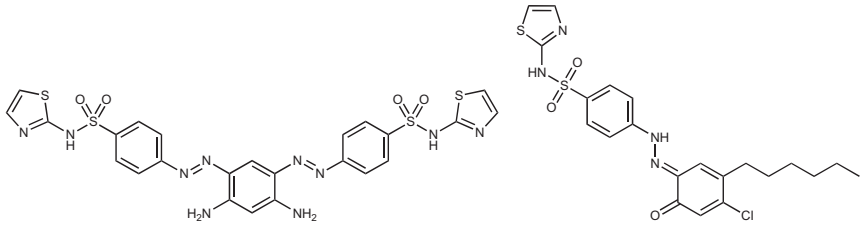
compounds in this series displayed noteworthy inhibitory activities including compound **13** ($K_i = 4.77$ μM). Molecular docking suggests that **13** spans the substrate-binding subsites of the enzyme, yet is not in direct contact with the catalytic triad.



13

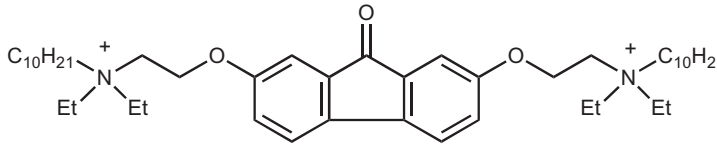
3.1.2 Noncompetitive inhibitors

To identify allosteric inhibitors of the protease activity of the related flavivirus WNV that bind the NS2B/NS3pro cofactor-binding site, a virtual screen of the NCI database of 275,000 compounds was performed. Two hits, **14** and **15**, inhibited DENV2 NS2B/NS3pro ($IC_{50} = 2.75$ and 2.04 μM, respectively).[52]



14 **15**

A high-throughput screen of DENV2 NS2b/NS3pro identified the dihydroxyfluoren-9-one derivative, **16** ($IC_{50} = 15.4$ μM), which showed comparable activity against all four DENV serotypes in infected BHK21 cells. Resistance mutations identified in the NS2B region conferred a 10-fold reduction in compound potency in enzyme inhibition and a 74-fold potency reduction in a replicon assay, suggestive of compound **16** binding to NS2B and interfering with the NS2B–NS3 interactions.[53]



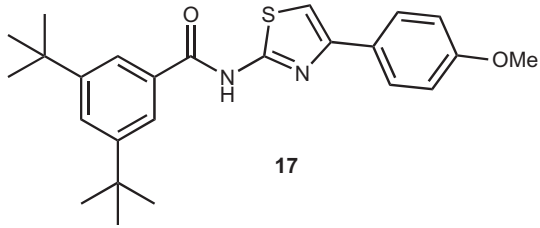
16

3.2. NS3 helicase

The C-terminal 440 residues of NS3 contains the RNA helicase, NTPase, and RTPase activities.[13] Few reports of inhibition of the C-terminal domain of NS3 (NTPase/helicase) of DENV have appeared, though available structural information[10,54–56] suggests the presence of a pocket for possible drug interaction.[56] Synthetic nucleoside derivatives tested against DENV NTPase/helicase showed only limited activity.[57]

3.3. NS4B

Though poorly understood, the NS4B protein is a small, hydrophobic, transmembrane protein that serves to anchor the viral replication machinery to the endoplasmic reticulum. Though lacking in intrinsic enzymatic activity, NS4B may represent a potential therapeutic target in the manner that the nonenzymic HCV NS5A protein has been exploited for therapeutic intervention.[58,59] Compound **17** was identified from a high-throughput screen of 1.8 million compounds against DENV2 replicon in A549 cells ($EC_{50} = 1.0$ μM).[60] Compound **17** was shown to have antiviral activity against all four DENV serotypes ($EC_{50} = 1.5$, 1.6, and 4.1 μM for DENV1, 3, and 4, respectively), but was not active against WNV or YFV. Mechanism of action studies indicated that **17** acts by suppression of viral RNA synthesis, while resistance selection studies identified mutations in the NS4B protein, notably NS4B P104L and A119T. These residues are conserved among all four DENV serotypes, but not in other flaviviruses. Furthermore, P104 and A119 are believed to be in a region of NS4B that locates in the ER membrane.[18]



17

3.4. NS5 polymerase

The C-terminus of the NS5 protein contains the RdRp (NS5pol), which, together with both viral and host proteins, synthesizes both positive- and negative-strand RNA. Again, available structural information and clinical success in targeting the HCV NS5 RdRp provide impetus for the discovery of both active-site and allosteric inhibitors.[61–64]

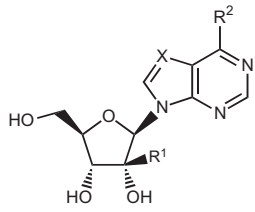
3.4.1 Nucleoside inhibitors of NS5pol

Inhibition of viral polymerases by nucleosides is an established, successful approach to treatment of viral infection in a variety of diseases, with nucleosides representing the largest single class of antiviral drugs. Nucleoside drugs exert their therapeutic efficacy following processing to the corresponding nucleotide triphosphate which then competes with endogenous nucleotides for incorporation into viral DNA or RNA.

2′-Methyl-7-deazaadenosine (MK0608) (**18**), a known inhibitor of the HCV NS5B polymerase, has also been shown to inhibit DENV in cell culture and shows efficacy in animal models of infection.[65] This result led to an expanded evaluation of 2′-modified nucleosides for anti-DENV activity.[66,67] Studies on 2′-modified adenosines demonstrated that only methyl (**19**) and ethynyl (**22**) substituents showed activity in DENV2-infected A549 cells (IC_{50}s = 1.1 and 1.4 μM, respectively). Ethyl (**20**), ethenyl (**21**), and propargyl (**23**) substituents were all effectively inactive (> 50 μM), as were 2′-ethynyl cytidine and guanines. To preclude deamination of 2′-C-substituted adenines, leading to inactive inosine derivatives, the C-7 purine nitrogen was replaced with carbon (compounds **24**–**27**). Compound **24** has emerged as a promising nucleoside lead, with submicromolar activity in DENV2-infected A549 cells (EC_{50} = 0.7 μM). Mechanism of action studies indicate that **24** inhibits DENV by blocking RNA synthesis. Further, both the 5′-*O*-mono- and triphosphates exhibit strong DENV inhibition in both cell culture and enzyme inhibition assays,[67] indicating **24** acts through blocking RNA chain elongation. The triphosphate of **24** demonstrates an NS5 polymerase K_i = 0.060 μM.[68] In addition, although unmodified **24** is observed in rat plasma, the mono-, di-, and triphosphate metabolites have been identified in blood cells. Pharmacokinetic studies indicated that **24** is orally bioavailable and has parameters consistent with twice-daily dosing.

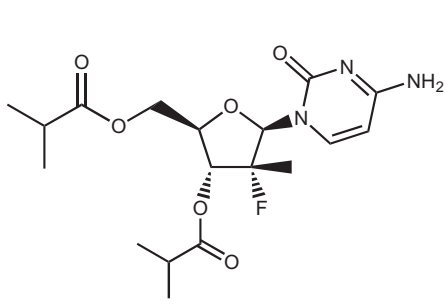
In DENV2-infected AG129 mice, a single dose of **24** was administered (25, 75, 150, and 300 mg/kg, p.o.) 12 h postinfection with DENV2.[67] At day 3, peak viremia was reduced in all dose groups, though the highest doses were not significantly superior to 75 mg/kg. In addition, a single 75-mg/kg

dose of **24** provided complete protection to mice infected with the lethal DENV2 D2S10 strain.[67] However, following 14-day toxicology studies in rats and dogs, a NOAEL could not be established for **24** in either species. Even at 10 mg/kg/day in rats and 1 mg/kg/day in dogs, notable toxicities were observed, including irreversible corneal damage.[66]

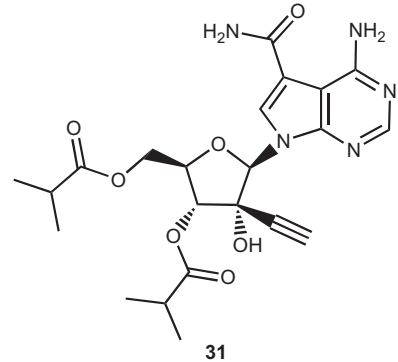


Compound	X	R^1	R^2
18	C-H	CH_3	NH_2
19	N	CH_3	NH_2
20	N	CH_2CH_3	NH_2
21	N	$CH{=}CH_2$	NH_2
22	N	C≡CH	NH_2
23	N	$C{\equiv}CCH_3$	NH_2
24	C-H	C≡CH	NH_2
25	C-F	C≡CH	NH_2
26	C-CN	C≡CH	NH_2
27	C-$CONH_2$	C≡CH	NH_2
28	N	H	CH_3
29	C-H	H	CH_3

Compound **27**, though slightly less potent in DENV2-infected A549 cells (EC_{50}: 2.6 μM), was also studied *in vivo*.[69] However, pharmacokinetic studies indicated very low oral bioavailability (*F* 1%) in mice and rats (*F* 2%). By analogy with the HCV polymerase inhibitor **30** (RG7128),[70] the di-isobutyryl derivative of **27** was evaluated.[69] Compound **31** exhibits enhanced cellular efficacy (EC_{50} = 0.69 μM), as well as improved oral bioavailability of **27** (*F* 32% and 10–13% in mice and rats, respectively). The prodrug **31** was not detected in plasma. Dosing 25 mg/kg of **31** in the mouse viremia model resulted in a 30-fold reduction in peak viremia; however, toxicological studies demonstrated weight loss and death in Wistar rats at doses of 30 and 75 mg/kg/day.[69]



30



31

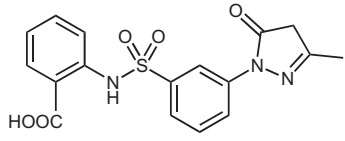
The 6-methyl adenosine analogs, compounds **28** and **29**, have been reported as inhibitors of the DENV2 replicon system in BHK21 cells (EC_{50} = 5.5 and 0.9 μM, respectively) and **29** showed even greater potency in DENV2-infected Vero cells (EC_{50} = 0.039–0.062 μM), though

cytotoxicity was noted in HeLa cells.[71] Thus, while anti-DENV efficacy and promising pharmacokinetics have been attained, further investigation of this class of antiviral is warranted in order to identify a viable drug candidate.

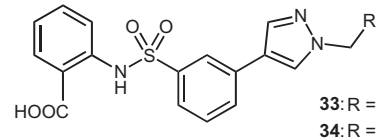
3.4.2 Non-nucleoside inhibitors of NS5pol

Non-nucleoside, allosteric inhibitors of HCV NS5B polymerase have garnered significant attention, with numerous molecules in clinical evaluation[63,64] and, by analogy, provide inspiration for analogous approaches to inhibit DENV NS5pol.[21]

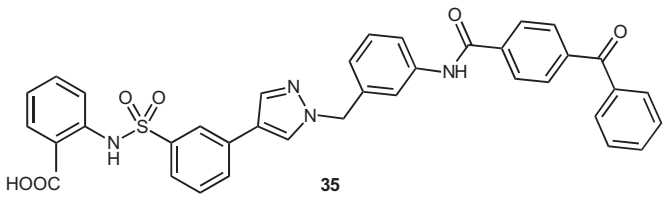
A high-throughput screen of more than 1 million compounds against the full-length NS5 protein reportedly resulted in a hit rate of 0.7%, from which compound **32** ($IC_{50} = 7.2$ μM) was identified as the basis for further optimization.[72,73] This optimization effort led to **33** ($IC_{50} = 0.7$ μM), which showed no activity toward human DNA polymerases, nor HCV NS5B, or WNV NS5 polymerases. Neither compound showed significant activity in infected cells. Further SAR around this series suggested that the carboxylate at the 2-position is essential for activity and that minor improvements might be attained by inclusion of an electron-withdrawing group at the aryl 5-position. A slight improvement in potency was obtained by replacing the *N*-benzyl group with *N*-2-naphthylmethyl, compound 34 (IC_{50} = 0.26 μM).[72] To better understand the binding location of this series, a photoaffinity experiment was performed with compound **35** ($IC_{50} = 1.5$ μM), which irreversibly inhibited NS5pol following UV irradiation. On the basis of the labeled residue (methionine 320/343) and structural analysis of the available X-ray structure and docking experiments, a binding mode was proposed at a site between the "finger" and "thumb" regions of the polymerase,[62] which would cause the occlusion of the RNA template tunnel. Compound **35** was notably more potent when added to the enzyme prior to the RNA template and weaker when added after. Cellular efficacy of these compounds was lacking.[73]



32



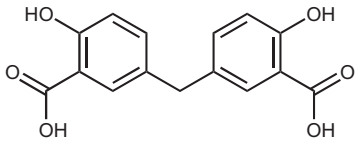
33: R = phenyl
34: R = 2-naphthyl



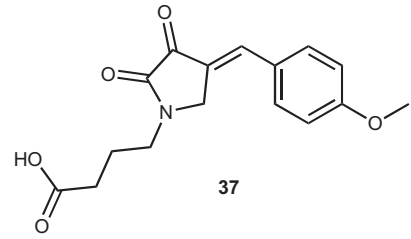
35

3.5. Methyltransferase inhibitors

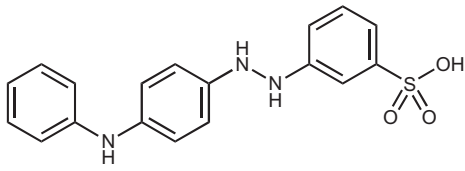
The N-terminus of the NS5 protein contains the methyl transferase domain (NS5mt) which serves to sequentially methylate both guanine N7 and adenosine ribose 2′-OH of the viral RNA cap. This is accomplished by methyl transfer from *S*-adenosyl methionine (SAM, **40**), affording *S*-adenosyl homocysteine (SAH, **41**) as the by-product.[74] X-ray crystal structures of DENV NS5mt have been reported[14,75–79] and have served as templates for virtual screening approaches to the discovery of new inhibitors.[80] Docking experiments at both the SAM and RNA sites led to the identification of confirmed inhibitors, two acting at the SAM site (**36** and **37** IC_{50}s = 9.5 and 4.4 μM, respectively) and two acting at the RNA site (**38** and **39** IC_{50}s = 7.1 and 4.9 μM, respectively).



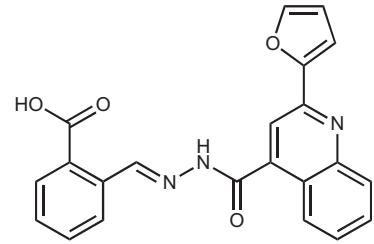
36



37



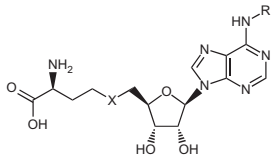
38



39

The RNA site has been observed to be shallow and solvent-exposed and may not represent a suitable site for drug interaction. In contrast, the SAM site is well defined, though the substrate is ubiquitous in methyltransferases and presents a potential selectivity challenge. Recently, a detailed analysis of flavivirus NS5 structures identified a conserved cavity adjacent to the adenine-binding pocket.[81] Synthesis of a series of SAH analogs identified a number of inhibitors of DENV3 NS5mt-mediated N7 and 2′-O-methylation, which also had activity against WNV NS5mt. Furthermore, N6-benzyl derivatives of **41** (compounds **42**–**44**) showed no inhibition

toward human RNA guanine-7-methyltransferase nor human DNA methyltransferase 1A, suggesting opportunities for selective inhibitor design.



Compound	X	R	K_i, N7-mt	K_i, 2′O-mt	K_i, hRNMT	K_i, DNMT
40	S^+-CH_3	H	NA	NA	NA	NA
41	S	H	3.2	0.57	4.5	6.2
42*	S	Bn	2.1	0.52	16	>50
43*	S	Bn(3-Me)	0.85	0.28	>50	>50
44*	S	Bn(3-Cl)	0.82	0.17	>50	>50

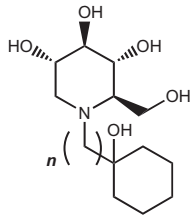
* epimeric at amino acid

4. HOST TARGETS

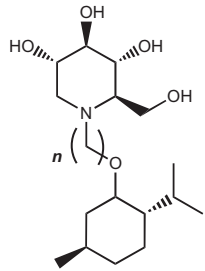
4.1. Maturation inhibitors

The viral E protein is a glycoprotein that is modified by the cellular sugar modifying machinery in the ER and Golgi complex. The sugar groups attached to the protein play a critical role during several steps in the viral life cycle.[82,83] Inhibition of the cellular enzymes responsible for the modifications on the E glycoprotein might be expected to reduce the production of new infectious virions and has a broad spectrum potential. Iminosugars have been used as competitive inhibitors for the ER α-glucosidases I and II which trim N-linked glycans on glycoproteins.[84] Until recently, the potential of iminosugars was limited by their low level of potency in combination with relatively high toxicity. However, deoxynojirimycin derivative **45** has improved activity ($IC_{50} = 2$ μM) and reduced cytotoxicity.[85] Derivatives **46** and **47** inhibited DENV2 with IC_{50}s of 0.075–0.1 μM. These molecules are also active against WNV and BVDV, albeit with lower potency.[86]

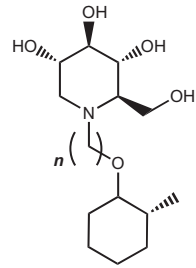
The iminosugar derivative **48** (DENV2 $IC_{50} = 1.1$ μM) was tested in the AG129 mouse DENV2 infection model.[87] When dosed p.o. at 100 mg/kg, the plasma concentration of **48**, 11 h postdosing, was approximately 5 μM ($5 \times IC_{50}$). Under these conditions, viral loads dropped approximately twofold. **48** was synergistic with ribavirin; however, α-glucosidase I activity was not detected suggesting that the activity might be mostly due to α-glucosidase II activity.[87] **48** also showed a survival benefit in a lethal DENV mouse challenge model.[88]



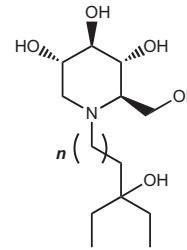
45 $n = 5$



46 $n = 6$

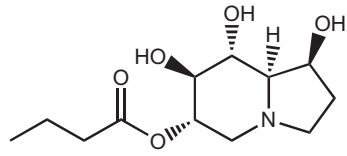


47 $n = 6$



48 $n = 5$

Celgosivir (6-*O*-butanoylcastanospermine, **49**), an inhibitor of α-glucosidase I and II, is known to affect folding of N-glycosylated proteins. DENV proteins prM, E, and NS1 are all believed to be N-glycosylated and **49** showed strong inhibition of DENV2-infected BHK21 fibroblasts ($IC_{50} = 0.20$ μM), with similar antiviral activity across all serotypes ($IC_{50} = 0.65$, 0.68, and 0.31 μM for DENV1, 3, and 4, respectively).[89] Further studies demonstrated both an effect on NS1 post-translational modification as well as effective inhibition of DENV2 replication in a subgenomic replicon system ($EC_{50} = 2.2$ μM). Compound **49** was evaluated in both a primary model of infection and in the lethal ADE model, in each case dosed at 50 mg/kg bid. In the latter model, mice are administered a DENV E protein cross-reactive antibody and challenged after 24 h with virus, resulting in 100% mortality after 5 days in the absence of treatment. Compound **49** proved effective in both models, providing complete protection when dosed from the time of infection. Furthermore, 50% survival in the lethal ADE model was obtained even when dosing of **49** was delayed 2 days postinfection.

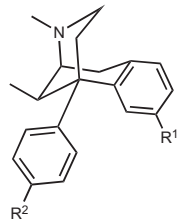


49

4.2. Translation inhibitors

Compound **50** is a molecule that was identified following a phenotypic screen with a 1.8 million compound library on DENV2-infected Huh-7 cells ($EC_{50} = 0.55$ μM).[90] The molecule inhibited the related YFV and WNV with EC_{90}s of 4.9 and 4.5 μM, respectively, while the EC_{90}s against the nonrelated WEEV and VSV were ~20 and >20 μM, respectively.

SAR efforts to improve the chemical characteristics of **50** resulted in **51**. Compound **51**, however, was found to nonselectively inhibit both viral and cellular protein translation. In short-term tissue culture studies, toxicity was limited; however, mice dosed at 75 mg/kg had severe side effects.

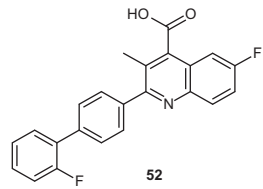


Compound	R^1	R^2
50*	OH	F
51	CN	H

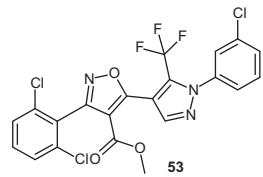
*racemic

4.3. Other mechanisms

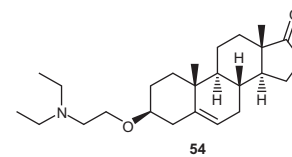
Other reportedly effective approaches to blocking DENV replication in cells have been demonstrated with the dihydroorotate dehydrogenase inhibitors brequinar, **52** ($EC_{50} = 0.078\ \mu M$),[91,92] and compound **53**,[93] the cholesterol uptake inhibitor **54** ($EC_{50} = 6.2\ \mu M$),[94] the deubiquinating enzyme inhibitor **55** ($EC_{50} = 40\ \mu M$),[95] the NTRK1/MAPKAPK5 kinase inhibitor **56** ($EC_{50} = 0.4\ \mu M$),[96] and compound **57** ($EC_{50} = 0.9\ \mu M$), with an unknown mechanism of action.[97]



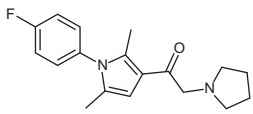
52



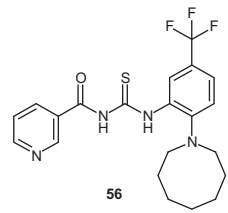
53



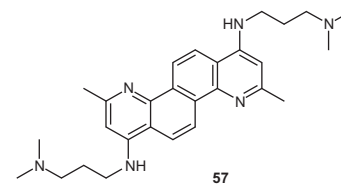
54



55



56



57

5. CONCLUSIONS

The rapid geographic spread of DENV over the past half century represents an emergence of an alarming global health threat. Recent clinical successes in the treatment of the related hepatitis C virus suggest that similar approaches to the treatment of DENV infection may represent fruitful

opportunities for drug discovery exploration. The well-established understanding of virus biology, coupled with the accessibility of viral protein target structures, has led to a significant application of virtual as well as high-throughput screens to identify chemical matter. Successes in animal models of infection also give cause for optimism, yet ideal candidate molecules for clinical development have yet to be reported.

REFERENCES

(1) Gubler, D.J. *Clin. Microbiol. Rev.* **1998**, *11*, 480.
(2) Guzman, A.; Istúriz, R.E. *Int. J. Antimicrob. Agents* **2010**, *36S*, S40.
(3) WHO. Dengue and severe dengue. Fact sheet number 117, January 2012. http://www.who.int/mediacentre/factsheets/fs117/en/ (accessed May 4, 2012).
(4) Gubler, D.J. *Am. J. Trop. Med. Hyg.* **1989**, *40*, 571.
(5) Iturbe-Ormaetxe, I.; Walker, T.; O'Neill, S.L. *EMBO Rep.* **2011**, *12*, 508.
(6) Harris, A.F.; Nimmo, D.; McKerney, A.R.; Kelly, N.; Scaife, S.; Donnelly, C.A.; Beech, C.; Petrie, W.D.; Alphey, L. *Nat. Biotechnol.* **2011**, *29*, 1034.
(7) Reiter, P.; Gubler, D.J. In *Dengue and Dengue Hemorrhagic Fever*; Gubler, D.J., Kuno, G., Eds.; CAB International: New York, 1997; p 425.
(8) Kummerer, B.M.; Rice, C.M. *J. Virol.* **2002**, *76*, 4773.
(9) Guo, J.T.; Hayashi, J.; Seeger, C. *J. Virol.* **2005**, *79*, 1343.
(10) Luo, D.; Xu, T.; Hunke, C.; Gruber, G.; Vasudevan, S.G.; Lescar, J. *J. Virol.* **2008**, *82*, 173.
(11) Wang, C.C.; Huang, Z.S.; Chiang, P.L.; Chen, C.T.; Wu, H.N. *FEBS Lett.* **2009**, *583*, 691.
(12) Li, H.; Clum, S.; You, S.; Ebner, K.E.; Padmanabhan, R. *J. Virol.* **1999**, *73*, 3108.
(13) Bartelma, G.; Padmanabhan, R. *Virology* **2002**, *299*, 122.
(14) Egloff, M.P.; Benarroch, D.; Selisko, B.; Romette, J.L.; Canard, B. *EMBO J.* **2002**, *21*, 2757.
(15) Ackermann, M.; Padmanabhan, R. *J. Biol. Chem.* **2001**, *276*, 39926.
(16) Lindenbach, B.D.; Rice, C.M. *J. Virol.* **1997**, *71*, 9608.
(17) Miller, S.; Kastner, S.; Krijnse-Locker, J.; Bühler, S.; Bartenschlager, R. *J. Biol. Chem.* **2007**, *282*, 8873.
(18) Miller, S.; Sparacio, S.; Bartenschlager, R. *J. Biol. Chem.* **2006**, *281*, 8854.
(19) Murphy, B.R.; Whitehead, S.S. *Annu. Rev. Immunol.* **2011**, *29*, 587.
(20) Durbin, A.P.; Whitehead, S.S. *Viruses* **1800**, *2011*, 3.
(21) Parkinson, T.; Pryde, D.C. *Future Med. Chem.* **2010**, *2*, 1181.
(22) Stevens, A.J.; Gahan, M.E.; Mahalingam, S.; Keller, P.A. *J. Med. Chem.* **2009**, *52*, 7911.
(23) Kaufmann, B.; Rossmann, M.G. *Microbes Infect.* **2011**, *13*, 1.
(24) Smit, J.M.; Moesker, B.; Rodenhuis-Zybert, I.; Wilschut, J. *Viruses* **2011**, *3*, 160.
(25) Modis, Y.; Ogata, S.; Clements, D.; Harrison, S.C. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 6986.
(26) Kampmann, T.; Yennamalli, R.; Campbell, P.; Stoermer, M.J.; Fairlie, D.P.; Kobe, B.; Young, P.R. *Antiviral Res.* **2009**, *84*, 234.
(27) Poh, M.K.; Shui, G.; Xie, X.; Shi, P.Y.; Wenk, M.R.; Gu, F. *Antiviral Res.* **2011**, *93*, 191.
(28) Wang, Q.-Y.; Patel, S.J.; Vangrevelinghe, E.; Xu, H.Y.; Rao, R.; Jaber, D.; Schul, W.; Gu, F.; Heudi, O.; Ma, N.L.; Poh, M.K.; Phong, W.Y.; Keller, T.H.; Jacoby, E.; Vasudevan, S.G. *Antimicrob. Agents Chemother.* **1823**, *2009*, 53.

(29) Yennamalli, R.; Subbarao, N.; Kampmann, T.; McGeary, R.P.; Young, P.R.; Kobe, B. *J. Comput. Aided Mol. Des.* **2009**, *23*, 333.
(30) Schmidt, A.G.; Yang, P.L.; Harrison, S.C. *J. Virol.* **2010**, *84*, 12549.
(31) Costin, J.M.; Jenwitheesuk, E.; Lok, S.-M.; Hunsperger, E.; Conrads, K.A.; Fontaine, K.A.; Rees, C.R.; Rossmann, M.G.; Isern, S.; Samudrala, R.; Michael, S.F. *PLoS Negl. Trop. Dis.* **2010**, *4*, e721.
(32) Nicholson, C.O.; Costin, J.M.; Rowe, D.K.; Lin, L.; Jenwitheesuk, E.; Samudrala, R.; Isern, S.; Michael, S.F. *Antiviral Res.* **2011**, *89*, 71.
(33) Hrobowski, Y.M.; Garry, R.F.; Michael, S.F. *Virol. J.* **2005**, *2*, 49.
(34) Alen, M.M.F.; Kaptein, S.J.F.; De Burghgraeve, T.; Balzarini, J.; Neyts, J.; Schols, D. *Virology* **2009**, *387*, 67.
(35) Acosta, E.G.; Castilla, V.; Damonte, E.B. *Cell. Microbiol.* **2009**, *11*, 1533.
(36) Kato, D.; Era, S.; Watanabe, I.; Arihara, M.; Sugiura, N.; Kimata, K.; Suzuki, Y.; Morita, K.; Hidari, K.I.P.J.; Suzuki, T. *Antiviral Res.* **2010**, *88*, 236.
(37) Erbel, P.; Schiering, N.; D'Arcy, A.; Renatus, M.; Kroemer, M.; Lim, S.P.; Yin, Z.; Keller, T.H.; Vasudevan, S.G.; Hommel, U. *Nat. Struct. Mol. Biol.* **2006**, *13*, 372.
(38) Lescar, J.; Luo, D.; Xu, T.; Sampath, A.; Lim, S.P.; Canard, B.; Vasudevan, S.G. *Antiviral Res.* **2008**, *80*, 94.
(39) Chen, K.X.; Njoroge, F.G. *Curr. Opin. Investig. Drugs* **2009**, *10*, 821.
(40) Mani, N.; Rao, B.G.; Kieffer, T.L.; Kwong, A.D. In *Antiviral Drug Strategies*; de Clercq, E., Ed.; Wiley-VCH Verlag, Weinheim 2011; p 307.
(41) Li, J.; Lim, S.P.; Beer, D.; Patel, V.; Wen, D.; Tumanut, C.; Tully, D.C.; Williams, J.A.; Jiricek, J.; Priestle, J.P.; Harris, J.L.; Vasudevan, S.G. *J. Biol. Chem.* **2005**, *280*, 28766.
(42) Schechter, I.; Berger, A. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157.
(43) Yin, Z.; Patel, S.J.; Wang, W.-L.; Wang, G.; Chan, W.-L.; Rao, K.R.R.; Alam, J.; Jeyaraj, D.A.; Ngew, X.; Patel, V.; Beer, D.; Lim, S.P.; Vasudevan, S.G.; Keller, T.K. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 36.
(44) Yin, Z.; Patel, S.J.; Wang, W.-L.; Wang, G.; Chan, W.-L.; Rao, K.R.R.; Alam, J.; Jeyaraj, D.A.; Ngew, X.; Patel, V.; Beer, D.; Lim, S.P.; Vasudevan, S.G.; Keller, T.K. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 40.
(45) Schüller, A.; Yin, Z.; Brian Chia, C.S.; Doan, D.N.P.; Kim, H.-K.; Shang, L.; Loh, T.P.; Hill, J.; Vasudevan, S.G. *Antiviral Res.* **2011**, *92*, 96–101.
(46) Knehans, T.; Schüller, A.; Doan, D.N.; Nacro, K.; Hill, J.; Güntert, P.; Madhusudhan, M.S.; Weil, T.; Vasudevan, S.G. *J. Comput. Aided Mol. Des.* **2011**, *25*, 263.
(47) Levitzki, A.; Mishani, E. *Annu. Rev. Biochem.* **2006**, *75*, 93.
(48) Nitsche, C.; Steuer, C.; Klein, C.D. *Bioorg. Med. Chem.* **2011**, *19*, 7318.
(49) Perola, E. *J. Med. Chem.* **2010**, *53*, 2986.
(50) Nitsche, C.; Behnam, M. A. M.; Steuer, C.; Klein, C. D. *Antiviral Res.* **2012**, *94*, 72.
(51) Tiew, K.-C.; Dou, D.; Teramoto, T.; Lai, H.; Alliston, K.R.; Lushington, G.H.; Padmanabhan, R.; Groutas, W.C. *Bioorg. Med. Chem.* **2012**, *20*, 1213.
(52) Shiryaev, S.A.; Cheltsov, A.V.; Gawlik, K.; Ratnikov, B.I.; Strongin, A.Y. *Assay Drug Dev. Technol.* **2011**, *9*, 69.
(53) Yang, C.-C.; Hsieh, Y.-C.; Lee, S.-J.-H.; Wu, S.-H.; Liao, C.-L.; Tsao, C.-L.; Chao, Y.-S.; Chern, J.-H.; Wu, C.-P.; Yueh, A. *Antimicrob. Agents Chemother.* **2011**, *55*, 229.
(54) Luo, D.; Wei, N.; Doan, D.N.; Paradkar, P.N.; Chong, Y.; Davidson, A.D.; Kotaka, M.; Lescar, J.; Vasudevan, S.G. *J. Biol. Chem.* **2010**, *285*, 18817.
(55) Xu, T.; Sampath, A.; Chao, A.; Wen, D.; Nanao, M.; Chene, P.; Vasudevan, S.G.; Lescar, J. *J. Virol.* **2005**, *79*, 10278.
(56) Sampath, A.; Xu, T.; Chao, A.; Luo, D.; Lescar, J.; Vasudevan, S.G. *J. Virol.* **2006**, *80*, 6686.

(57) Bretner, M.; Schalinski, S.; Hang, A.; Lang, M.; Schmitz, H.; Baier, A.; Behrens, S.-E.; Kulikowski, T.; Borowski, P. *Antiviral Chem. Chemother.* **2004**, *15*, 35.
(58) Gao, M.; Nettles, R.E.; Belema, M.; Snyder, L.B.; Nguyen, V.N.; Fridell, R.A.; Serrano-Wu, M.H.; Langley, D.R.; Sun, J.-H.; O'Boyle, D.R., II; Lemm, J.A.; Wang, C.; Knipe, J.O.; Chien, C.; Colonno, R.J.; Grasela, D.M.; Meanwell, N.A.; Hamann, L.G. *Nature* **2010**, *465*, 96.
(59) Lemm, J.A.; Leet, J.E.; O'Boyle, D.R.; Romine, J.L.; Huang, X.S.; Schroeder, D.R.; Alberts, J.; Cantone, J.L.; Sun, J.-H.; Nower, P.T.; Martin, S.W.; Serrano-Wu, M.H.; Meanwell, N.A.; Snyder, L.B.; Gao, M. *Antimicrob. Agents Chemother.* **2011**, *55*, 3795.
(60) Xie, X.; Wang, Q.-Y.; Xu, H.Y.; Qing, M.; Kramer, L.; Yuan, Z.; Shi, P.-Y. *J. Virol.* **2011**, *85*, 11183.
(61) Rawlinson, S.M.; Pryor, M.J.; Wright, P.J.; Jans, D.A. *Curr. Drug Targets* **2006**, *7*, 1623.
(62) Choi, K.H.; Rossmann, M.G. *Curr. Opin. Struct. Biol.* **2009**, *19*, 746.
(63) Beaulieu, P.L. *Curr. Opin. Investig. Drugs* **2007**, *8*, 614.
(64) Deore, R.R.; Chern, J.W. *Curr. Med. Chem.* **2010**, *17*, 3806.
(65) Schul, W.; Liu, W.; Xu, H.-Y.; Flamand, M.; Vasudevan, S.G. *J. Infect. Dis.* **2007**, *195*, 665.
(66) Yin, Z.; Chen, Y.L.; Schul, W.; Wang, Q.Y.; Gu, F.; Duraiswamy, J.; Kondreddi, R.R.; Niyomrattanakit, P.; Lakshminarayana, S.B.; Goh, A.; Xu, H.Y.; Liu, W.; Liu, B.; Lim, J.Y.H.; Ng, C.Y.; Qing, M.; Lim, C.C.; Yip, A.; Wang, G.; Chan, W.L.; Tan, H.P.; Lin, K.; Zhang, B.; Zou, G.; Bernard, K.A.; Garrett, C.; Beltz, K.; Dong, M.; Weaver, M.; He, H.; Pichota, A.; Dartois, V.; Keller, T.H.; Shi, P.-Y. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 20435.
(67) Chen, Y.-L.; Yin, Z.; Duraiswamy, J.; Schul, W.; Lim, C.C.; Liu, B.; Xu, H.Y.; Qing, M.; Yip, A.; Wang, G.; Chan, W.L.; Tan, H.P.; Lo, M.; Liung, S.; Kondreddi, R.R.; Rao, R.; Gu, H.; He, H.; Keller, T.H.; Shi, P.-Y. *Antimicrob. Agents Chemother.* **2010**, *54*, 2932.
(68) Latour, D.R.; Jekle, A.; Javanbakht, H.; Henningsen, R.; Gee, P.; Lee, I.; Tran, P.; Ren, S.; Kutach, A.K.; Harris, S.F.; Wang, S.M.; Lok, S.J.; Shaw, D.; Li, J.; Heilek, G.; Klumpp, K.; Swinney, D.C.; Deval, J. *Antiviral Res.* **2010**, *87*, 213.
(69) Chen, Y.-L.; Yin, Z.; Lakshminarayana, S.B.; Qing, M.; Schul, W.; Duraiswamy, J.; Kondreddi, R.R.; Goh, A.; Xu, H.Y.; Yip, A.; Liu, B.; Weaver, M.; Dartois, V.; Keller, T.H.; Shi, P.-Y. *Antimicrob. Agents Chemother.* **2010**, *54*, 3255.
(70) Cole, P.; Castaner, R.; Bolos, J. *Drugs Future* **2009**, *34*, 282.
(71) Wu, R.; Smidansky, E.D.; Oh, H.S.; Takhampunya, R.; Padmanabhan, R.; Cameron, C.E.; Peterson, B.R. *J. Med. Chem.* **2010**, *53*, 7958.
(72) Yin, Z.; Chen, Y.-L.; Kondreddi, R.R.; Chan, W.L.; Wang, G.; Ng, R.H.; Lim, J.Y.H.; Lee, W.Y.; Jeyaraj, D.A.; Niyomrattanakit, P.; Wen, D.; Chao, A.; Glickman, J.F.; Voshol, H.; Mueller, D.; Spanka, C.; Dressler, S.; Nilar, S.; Vasudevan, S.G.; Shi, P.-Y.; Keller, T.H. *J. Med. Chem.* **2009**, *52*, 7934.
(73) Niyomrattanakit, P.; Chen, Y.-L.; Dong, H.; Yin, Z.; Qing, M.; Glickman, J.F.; Lin, K.; Mueller, D.; Voshol, H.; Lim, J.Y.H.; Nilar, S.; Keller, T.H.; Shi, P.-Y. *J. Virol.* **2010**, *84*, 5678.
(74) Liu, L.; Dong, H.; Chen, H.; Zhang, J.; Ling, H.; Li, Z.; Shi, P.Y.; Li, H. *Front. Biol.* **2010**, *5*, 286.
(75) Benarroch, D.; Egloff, M.-P.; Mulard, L.; Guerreiro, C.; Romette, J.-L.; Canard, B. *J. Biol. Chem.* **2004**, *279*, 35638.
(76) Zhou, Y.; Ray, D.; Zhao, Y.; Dong, H.; Ren, S.; Li, Z.; Guo, Y.; Bernard, K.A.; Shi, P.-Y.; Li, H. *J. Virol.* **2007**, *81*, 3891.
(77) Egloff, M.-P.; Decroly, E.; Malet, H.; Selisko, B.; Benarroch, D.; Ferron, F.; Canard, B. *J. Mol. Biol.* **2007**, *372*, 723.
(78) Geiss, B.J.; Thompson, A.A.; Andrews, A.J.; Sons, R.L.; Gari, H.H.; Keenan, S.M.; Peersen, O.B. *J. Mol. Biol.* **2009**, *385*, 1643.

(79) Yap, L.J.; Luo, D.; Chung, K.Y.; Lim, S.P.; Bodenreider, C.; Noble, C.; Shi, P.-Y.; Lescar, J. *PLoS One* **2010**, *5*, e12836.
(80) Podvinec, M.; Lim, S.P.; Schmidt, T.; Scarsi, M.; Wen, D.; Sonntag, L.-S.; Sanschagrin, P.; Shenkin, P.S.; Schwede, T. *J. Med. Chem.* **2010**, *53*, 1483.
(81) Lim, S.P.; Sonntag, L.S.; Noble, C.; Nilar, S.H.; Ng, R.H.; Zou, G.; Monaghan, P.; Chung, K.Y.; Dong, H.; Liu, B.; Bodenreider, C.; Lee, G.; Ding, M.; Chan, W.L.; Wang, G.; Jian, Y.L.; Chao, A.T.; Lescar, J.; Yin, Z.; Vedananda, T.R.; Keller, T.H.; Shi, P.-Y. *J. Biol. Chem.* **2011**, *286*, 6233–6240.
(82) Mondotte, J.A.; Lozach, P.-Y.; Amara, A.; Gamarnik, A.V. *J. Virol.* **2007**, *81*, 7136.
(83) Bryant, J.E.; Calvert, A.E.; Mesesan, K.; Crabtree, M.B.; Volpe, K.E.; Silengo, S.; Kinney, R.M.; Huang, C.Y.-H.; Miller, B.R.; Roehrig, J.T. *Virology* **2007**, *366*, 415.
(84) Courageot, M.-P.; Frenkiel, M.-P.; Duarte Dos Santos, C.; Deubel, V.; Desprès, P. *J. Virol.* **2000**, *74*, 564.
(85) Gu, B.; Mason, P.; Wang, L.; Norton, P.; Bourne, N.; Moriarty, R.; Mehta, A.; Despande, M.; Shah, R.; Block, T. *Antivir. Chem. Chemother.* **2007**, *18*, 49.
(86) Chang, J.; Wang, L.; Ma, D.; Qu, X.; Guo, H.; Xu, X.; Mason, P.M.; Bourne, N.; Moriarty, R.; Gu, B.; Guo, J.-T.; Block, T.M. *Antimicrob. Agents Chemother.* **2009**, *53*, 1501.
(87) Chang, J.; Schul, W.; Butters, T.D.; Yip, A.; Liu, B.; Goh, A.; Lakshminarayana, S.B.; Alonzi, D.; Reinkensmeier, G.; Pan, X.; Qu, X.; Weidner, J.M.; Wang, L.; Yu, W.; Bourne, N.; Kinch, M.A.; Rayahin, J.E.; Moriarty, R.; Xu, X.; Shi, P.-Y.; Guo, J.-T.; Block, T.M. *Antiviral Res.* **2011**, *89*, 26.
(88) Chang, J.; Schul, W.; Yip, A.; Xu, X.; Guo, J.-T.; Block, T.M. *Antiviral Res.* **2011**, *92*, 369–371.
(89) Rathore, A.P.S.; Paradkar, P.N.; Watanabe, S.; Tan, K.H.; Sung, C.; Connolly, J.E.; Low, J.; Ooi, E.E.; Vasudevan, S.G. *Antiviral Res.* **2011**, *92*, 453.
(90) Wang, Q.-Y.; Kondreddi, R.R.; Xie, X.; Rao, R.; Nilar, S.; Xu, H.Y.; Qing, M.; Chang, D.; Dong, H.; Yokokawa, F.; Lakshminarayana, S.B.; Goh, A.; Schul, W.; Kramer, L.; Keller, T.H.; Shi, P.-Y. *Antimicrob. Agents Chemother.* **2011**, *55*, 4072.
(91) Tan, Y. H.; Driscoll, J. S.; Mui Mui, S. Patent Application WO 2001/024785, **2001**.
(92) Qing, M.; Zou, G.; Wang, Q.-Y.; Xu, H.Y.; Dong, H.; Yuan, Z.; Shi, P.-Y. *Antimicrob. Agents Chemother.* **2010**, *54*, 3686.
(93) Wang, Q.-Y.; Bushell, S.; Qing, M.; Xu, H.Y.; Bonavia, A.; Nunes, S.; Zhou, J.; Poh, M.K.; Florez de Sessions, P.; Niyomrattanakit, P.; Dong, H.; Hoffmaster, K.; Goh, A.; Nilar, S.; Schul, W.; Jones, S.; Kramer, L.; Compton, T.; Shi, P.-Y. *J. Virol.* **2011**, *85*, 6548.
(94) Poh, M.K.; Shui, G.; Xie, X.; Shi, P.Y.; Wenk, M.R.; Gu, F. *Antiviral Res.* **2012**, *93*, 191.
(95) Nag, D.K.; Finley, D. *Virus Res.* **2012**, *165*, 103.
(96) Anwar, A.; Hosoya, T.; Leong, K.M.; Onogi, H.; Okuno, Y.; Hiramatsu, T.; Koyama, H.; Suzuki, M.; Hagiwara, M.; Garcia-Blanco, M.A. *PLoS One* **2011**, *6*, e23246.
(97) Aman, M.J.; Kinch, M.S.; Warfield, K.; Warren, T.; Yunus, A.; Enterlein, S.; Stavale, E.; Wang, P.; Chang, S.; Tang, Q.; Porter, K.; Goldblatt, M.; Bavari, S. *Antiviral Res.* **2009**, *83*, 245.